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QTL detection for eating quality of cooked rice in a population of chromosome segment substitution lines

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Abstract The genetic mechanism underlying six palatability properties of cooked rice and three physico-chemical traits was dissected in 66 BC_3F_2 chromosome segment substitution lines (CSSLs), using a complete linkage map in three successive years. The CSSLs showed transgressive segregation for all traits studied. Significant correlation was detected among most palatability traits. A total of 25 QTLs for the nine traits were identified on nine chromosomes, and many QTLs affecting different quality traits were mapped in the same regions. Six QTLs—*qLT-8* for luster, *qTD-6* and *qTD-8* for tenderness, *qIVOE-6* and *qIVOE-8* for integrated value of organoleptic evaluation, and *qAC-8* for amylose content—were repeatedly detected across the 3 years. Phenotypic values were significantly different between the recurrent parent, cultivar Asominori, and the CSSLs harboring any of the six QTL alleles across the three environments, indicating that these six QTLs were non-environment-specific and could be used for marker-assisted selection in rice quality improvement.

Introduction

Rice grain quality is a complex character composed of many components such as milling, appearance, nutritional, cooking, and eating quality. Among these quality properties, consumers pay more attention to the fine appearance and high eating quality. Therefore, these are major goals in rice quality improvement (Huang et al. 1998). Knowledge accumulated in the past decades indicates that rice eating quality is directly related to three physico-chemical properties, namely, amylose content [(AC) Juliano 1985], gel consistency [(GC) Cagampang et al. 1973] and gelatinization temperature [(GT) Little et al. 1958]. So far, most research on cooking and eating quality has focused on AC, GC, and GT. Many QTLs were detected, of which major QTLs for AC and GC were allelic or tightly linked to the Waxy (*Wx*) gene on chromosome 6 (Lanceras et al. 2000), and major QTLs for GT were allelic or closely linked to the *alk* gene on chromosome 6 (He et al. 1999; Lanceras et al. 2000). Furthermore, it was also found that AC, GC, and GT were controlled by the linked genomic region near the *Wx* locus (Tan et al. 1999).

These physico-chemical traits have limitations as indices to identify whether a rice variety will cook to be dry and fluffy or soft and sticky. High physico-chemical quality is not fully equivalent to better eating quality (Bett-Garber et al. 2001). Palatability properties, including luster (LT), scent (ST), tenderness (TD), viscosity (VC), elasticity (EL), and the integrated value of organoleptic evaluation (IVOE), can be used to describe eating quality of cooked rice appropriately and are more effective than physico-chemical properties. In recent years, the relationships among these physico-chemical properties, rapid viscosity analyzer (RVA) profile of endosperm starch and eating quality, were evaluated (Zhang et al. 2002; Zhu et al. 2001; Champagne et al. 1999). However, no reports were found concerning genetic analysis for palatability properties of cooked rice. The reasons are perhaps that analytical methods for evaluating texture or flavor components are more difficult and not routinely used.

In this study, a direct method for evaluating eating quality was established based on Yamamoto et al. (1995), Champagne et al. (1999), and the National

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Standard of People's Republic of China [(NSPRC) 1999]. A chromosome segment substitution line (CSSL) population derived from cultivar Asominori (*japonica*)/IR24 (*indica*) backcrossed to Asominori and composed of 66 CSSLs was used for QTL identification. The CSSLs have several advantages over primary mapping populations such as F_2, F_3, recombinant inbred line (RIL), and double haploids in conducting QTL studies for complex traits. First, each CSSL carries a single or fewer donor segments in the near-isogenic background of a recurrent genotype. Interactions between donor alleles are limited to those between genes on homozygous substituted tracts, reducing the effects of interferences from genetic background (Howell et al. 1996; Yano 2001). Second, high-resolution mapping of putative QTLs as Mendelian factors and further map-based cloning will be feasible in many plants, using secondary F_2 population derived from a cross between a QTL-CSSL and the recurrent parent (Eshed and Zamir 1995; Frary et al. 2000; Takahashi et al. 2001; Yano et al. 2000). In addition, a secondary F_2 population between different target CSSLs can be used to precisely detect and confirm epistasis between QTLs (Lin et al. 2000; Yamamoto et al. 2000). Finally, the CSSLs can be used for simultaneous identification, mapping, and transfer of multiple desirable QTLs for target traits, especially for QTL pyramiding in plant breeding programs (Li 2001). The objectives of this study were to find non-environment-specific QTLs for eating quality of cooked rice, which should be helpful in marker-assisted selection (MAS) of high eating quality rice varieties and map-based cloning of desirable QTLs, and to elucidate the relationship between the physico-chemical properties and palatability characters of cooked rice.

Materials and methods

Population development

A total of 71 RILs derived from a cross between Asominori and IR24, were developed by Tsunematsu et al. (1996), using the single-seed descent method. To produce a series of CSSLs with the Asominori genetic background, 19 desirable RILs carrying more than 60% of Asominori genotypes were successively backcrossed with Asominori, but without selection until the BC_3F_1 generation. A total of 66 promising plants were selected from 268 BC_3F_1 plants by a whole-genome survey at 116 RFLP loci and MAS (Fig. 1) and nominated as $CSSL_1$–$CSSL_{66}$. These 66 CSSLs represented the whole IR24 genome except for the C1468–G1015 region (9.8 cM) on chromosome 3. This work was completed in Japan (Kubo et al. 1999).

In the summers of 2000, 2001, and 2002, Asominori, IR24, 66 CSSLs, and the control variety, cultivar Wuyujing 3, were grown in the experimental field of the Jiangsu Academy of Agricultural Sciences, Nanjing, China. Each entry was planted in a ten rows × ten plants array, with randomized block design and two

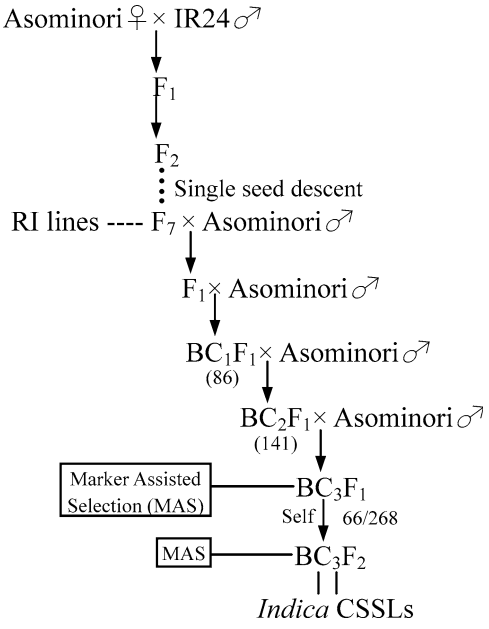

Fig. 1 The strategy for constructing the chromosome segment substitution lines population with the genetic background of a *japonica* variety, cultivar Asominori (quoted from Kubo et al. 1999)

replications. At maturity, each entry was harvested in bulk. After drying, grains were stored at room temperature for 3 months, and then polished and tested for palatability properties.

Phenotypic data collection

Rice was polished and cooked as described by Yamamoto et al. (1995) and Champagne et al. (1999), with modifications. Briefly, rice of each accession was polished separately. Before cooking, polished rice was washed four to five times with tap water and soaked in water for 30 min. Each entry was cooked in an MB-YH16 model rice cooker (Midea, Guangdong, China) at a ratio of polished rice:water = 1:1.3 (w/w). Seven CSSLs and two control samples were degusted each time. One check was assigned to a fixed cooker (cooker no. 1), and the other was randomly assigned to normalize the data. According to the methods of Yamamoto et al. (1995) and the NSPRC (1999), 12 males and 12 females were selected from 60 volunteers as panel members based on their abilities to sensitively identify palatability difference of cooked rice among varieties.

Of six degusting properties, LT and ST were scored by visual inspection and smelling of cooked rice as soon as the cookers were uncovered. TD, VC, and EL were estimated by chewing. Finally, the palatability was evaluated based on overall quality of the above five attributes. For each property, seven scores including −3, −2, −1, 0, +1, +2, and +3 were designed, representing worst, worse, bad, the same, good, better and best, respectively, when compared with the control variety

(Yamamoto et al. 1995; Bett-Garber et al. 2001). A scatterplot consisting of these scores was produced for each panelist and attribute. The phenotypic value of LT, ST, TD, VC, and EL for control was 15, respectively, and that of IVOE was 50. Conversion of degusting scores into organoleptic scores (the phenotypic values) was conducted based on the following formula (Yamamoto et al. 1995; NSPRC 1999):

$$y_i = \left[\left(\sum_j \frac{x_j}{24}\right) \times 5\right] + 15$$

$$Z = \left[\left(\sum_j \frac{x_j}{24}\right) \times 15\right] + 50$$

where, y_i denoted value of the i^{th} palatability property ($i = 1, 2, 3, 4$ and 5) denoted LT, ST, TD, VC, and EL, respectively; z denoted value of IVOE; x_j denoted degusting class of the i^{th} degusting property by the j^{th} organoleptic member ($j = 1, 2, \ldots, 24$).

Amylose content of each accession was determined with the simplified method developed by Juliano (1971), the GC with the method of Cagampang et al. (1973), the GT (alkali spreading score, ASS) with the procedure of Little et al. (1958).

The above nine traits were estimated with three replications for each sample.

Linkage map construction

Genomic DNA was extracted by the CTAB method (Murray and Thompson 1980). DNA clones, mapped by Tsunematsu et al. (1996), were used as DNA probes. DNA labeling, hybridization, and signal detection were conducted using the ECL detection system. For the whole-genome survey, 116 RLFP markers for the CSSLs scattered over the framework map were used in the BC_3F_1 generation. A linkage map was constructed with 85 RFLP markers evenly distributing on 12 chromosomes by using MAPMAKER/EXP, version 3.0 (Lander et al. 1987), and this map had a total length of 1,275.4 cM, with an average distance of 15.0 cM between adjacent markers. This work was done in Japan (Kubo et al. 1999).

Data analysis

According to classical quantitative genetics theory (Falconer 1981), the phenotypic value of a CSSL (y_{jhk}) in a specific environment can be described by the following genetic model:

$$y_{jhk} = \mu + G_j + Y_h + GY_{jh} + e_{jhk}$$

where y_{jhk} is the phenotypic value of a quantitative trait measured on the j^{th} CSSL ($j = 1, 2, \ldots, 66$) of the k^{th} field replication in the h^{th} year, μ is the CSSLs population mean; G_j is the genotypic effect (fixed) of the j^{th} CSSL, $Y_h \sim N(0, \sigma^2_Y)$ is the random effect of the h^{th} year ($h = 1, 2, 3$); $GY_{jh} \sim N(0, \sigma^2_{GY})$ is the interaction effect between the j^{th} CSSL and the h^{th} year, and $e_{jhk} \sim N(0, \sigma^2_e)$ the random residual effect.

The QTL parameters were estimated with composite interval mapping (CIM), a combination of simple interval mapping and multiple linear regression (Zeng 1994). For QTL analysis on a segment between markers i and $i+1$, the statistics model is:

$$y_j = b_0 + b_i x_{ij} + \sum_{k \neq i,i+1} b_k x_{kj} + \varepsilon_j$$

where y_j is the trait value for individual j, b_0 is the intercept of the model; b_i is the genetic effect of the putative QTL located between markers i and $i+1$; x_{ij} is the dummy variable taking 1 for maker genotype $AABB$ and 0 for $aabb$ 1 with a probability of $1-r_1/r = 1-\rho$ and 0 with a probability $r_1/r = \rho$ for maker genotype $aaBB$; 1 with probability of ρ and 0 with a probability of $1-\rho$ or marker genotype $AAbb$; r is the recombination fraction between the two markers; and r_i is the recombination between the first maker and the QTL. b_k is the partial regression coefficient of the trait value on marker k; x_{kj} is the dummy variable for marker k and individual j taking 1 if the marker has genotype AA and 0 for aa. ϵ_j is a residual from the model, normally distributed with mean zero and variance σ^2.

To obtain the empirical thresholds of the experiment, 1,000 permutations were run by randomly shuffling the trait values (Churchill and Doerge 1994). Thus, the LOD = 2.3 (the mean of the LOD values of all traits) was used for claiming significant main-effect QTLs in this study. QTL mapping was performed based on the data in each year using the JMP, version 3.1, software package (SAS Institute 1994).

In addition, a t-test was employed to determine the presence of significant differences between the phenotypic values of Asominori and those of CSSLs harboring target QTL alleles.

Results

Variation of phenotypic traits

Table 1 shows the phenotypic variation of the CSSLs and their parents for nine quality traits from 2000 to 2002. Significant differences were observed on all the traits between the parents. For example, Asominori had higher phenotypic values than IR24 for six traits including LT, TD, VC, EL, IVOE, and GC, but lower for ST, AC, and GT in 3 years. Meanwhile, the CSSLs showed continuous distribution with transgressive segregation for these traits. The mean and coefficients of variation of the CSSLs both remained largely consistent for each quality trait in 3 years, except that GT showed a much greater variation in 2000. In addition, the ANOVA indicated that variances among the genotypes (the CSSLs), the three environ-

Table 1 Summary statistics of phenotypic performance of the chromosome segment substitution lines (*CSSLs*) population and its parents for nine quality traits from 2000 to 2002

Traits and years[b]	Variation				
	Parents		The CSSLs population		
	Asominori	IR24	Mean ± SD[a]	Range	CV[a] (%)
LT					
2000	11.7	8.3	11.6 ± 1.5	7.8–14.9	12.9
2001	11.9	9.8	11.6 ± 1.3	7.5–14.8	11.6
2002	11.4	9.1	11.7 ± 1.5	7.7–15.6	13.0
ST					
2000	13.5	14.2	13.1 ± 0.7	10.2–14.4	5.5
2001	13.4	14.6	14.3 ± 0.7	12.7–15.9	4.9
2002	13.1	14.6	13.6 ± 1.0	10.7–15.5	7.5
TD					
2000	11.6	10.3	12.1 ± 1.9	5.4–17.1	15.9
2001	12.6	11.3	12.2 ± 1.4	7.1–15.2	11.6
2002	12.1	10.4	10.7 ± 1.6	6.1–14.6	15.0
VC					
2000	11.5	9.1	12.1 ± 1.0	8.7–15.0	8.2
2001	12.9	11.2	11.3 ± 1.4	7.1–14.1	12.6
2002	11.4	10.6	11.7 ± 1.2	8.3–14.4	10.1
EL					
2000	14.3	11.3	12.0 ± 1.4	7.7–15.9	11.6
2001	12.9	11.8	13.3 ± 1.2	8.9–16.2	9.1
2002	13.0	10.1	11.5 ± 1.5	8.2–14.5	12.8
IVOE					
2000	36.3	31.7	35.7 ± 6.8	17.1–52.7	19.1
2001	38.5	34.0	36.5 ± 5.4	18.2–49.3	14.8
2002	34.8	25.9	32.1 ± 6.4	16.9–51.5	20.0
AC					
2000	14.6	16.9	14.7 ± 1.2	9.9–17.0	8.2
2001	15.1	17.3	14.5 ± 1.1	12.3–17.8	7.7
2002	15.3	17.4	16.4 ± 0.9	14.9–19.0	5.6
GT					
2000	4.1	5.1	3.9 ± 0.8	2.7–5.9	21.5
2001	4.8	5.3	5.1 ± 0.1	4.3–5.4	2.6
2002	4.9	5.4	5.2 ± 0.1	3.9–5.4	2.2
GC					
2000	58.7	51.4	55.0 ± 4.3	36.4–59.2	7.7
2001	58.7	47.9	53.5 ± 6.2	36.8–63.9	11.5
2002	57.5	50.8	52.3 ± 6.4	33.7–63.4	12.1

[a]See "Materials and methods" for details of parameter calculations. *SD* Standard derivation , *CV* coefficient of variation
[b]*LT* Luster, *ST* scent, *TD* tenderness, *VC* viscosity, *EL* elasticity, *IVOE* integrated value of organoleptic evaluation, *AC* amylose content (%), *GT* gelatinization temperature, *GC* gel consistency (mm)

ments, and the G×E interactions were significant for all the traits. The environmental variances accounted for over 75% of the total phenotypic variation for nine quality traits except LT and GC (Table 2).

Correlations

All traits except EL, GT, and GC show significant correlations in the CSSLs (Table 3). Highly significant positive correlations were observed between any two traits among IVEO, LT, TD, AC, and ST, in which the strongest correlation was observed between IVOE and TD ($r = 0.836$**) and the lowest correlation between LT and ST ($r = 0.326$**).

Table 2 Analysis of variance for rice eating quality traits in the CSSLs population across 3 years

Traits and Sources	*df*	MS	*F*-values
LT			
Year	2	0.48	3.44*
CSSL	64	8.92	64.18**
Year × CSSL	128	1.86	13.42**
ST			
Year	2	41.57	370.31**
CSSL	64	2.30	20.46**
Year × CSSL	128	0.89	7.93**
TD			
Year	2	86.46	333.02**
CSSL	64	11.90	45.84**
Year × CSSL	128	2.17	8.36**
VC			
Year	2	24.26	178.21**
CSSL	64	5.77	42.41**
Year × CSSL	128	1.49	10.93**
EL			
Year	2	106.32	370.79**
CSSL	64	6.49	22.63**
Year × CSSL	128	2.31	8.06**
IVOE			
Year	2	706.80	285.00**
CSSL	64	184.40	74.36**
Year × CSSL	128	22.81	9.20**
AC			
Year	2	153.36	564.66**
CSSL	64	3.98	14.65**
Year × CSSL	128	1.37	5.05**
GT			
Year	2	67.61	614.70**
CSSL	64	0.57	5.20**
Year × CSSL	128	0.45	4.10**
GC			
Year	2	230.45	112.49**
CSSL	64	143.14	69.88**
Year × CSSL	128	24.74	12.08**

Significance levels: *$P \le 0.05$, **$P \le 0.001$

Table 3 Phenotypic correlations among sensory attributes and physico-chemical properties from 66 CSSLs with the Asominori genetic background based on the mean values in 3 years

Traits	LT	ST	TD	VC	EL	IVOE	AC	GT
ST	0.326**							
TD	0.726**	0.468**						
VC	0.558**	0.261*	0.687**					
EL	0.272*	NS[a]	NS	NS				
IVOE	0.794**	0.413**	0.836**	0.686**	0.519**			
AC	0.364**	0.368**	0.525**	0.442**	NS	0.546**		
GT	−0.307*	NS	NS	NS	NS	−0.278*	NS	
GC	NS	0.264*	NS	NS	NS	NS	NS	NS

Significance levels: *$P \le 0.05$, **$P \le 0.001$
[a]*NS* Non-significant

QTL analysis

A total of 25 QTLs influencing nine quality traits were identified and mapped on nine chromosomes except 2, 7, and 10, with LOD values of 2.3–8.3 (Table 4; Fig. 2).

Out of 13 QTLs affecting six palatability properties, four QTLs (*qLT-6*, *qTD-6*, *qVC-6*, and *qIVOE-6*) were

Table 4 QTL locations and biometrical parameters for nine quality traits in individual years. *AE* QTL additive effect, *PVE* percentage of phenolyph variation explained

Traits/QTL (chromosome no.)	Marker interval	Years	LOD	AE[a]	PVE[a]	Allele[b]
LT						
qLT-8 (8)	G1149–XNpb41	2000	2.3	1.5	17.0	I
		2001	4.2	1.4	28.5	I
		2002	3.6	1.4	18.6	I
qLT-4 (4)	C445–Ky4	2001	2.7	−1.8	10.9	A
qLT-6 (6)	XNpb209–C688	2001	2.9	−1.3	20.8	A
		2002	2.3	−1.4	18.4	A
ST						
qST-8 (8)	G1149–XNpb41	2000	3.6	−1.0	22.1	A
		2002	3.7	−1.3	33.1	A
qST-4 (4)	C445–Ky4	2000	2.3	−1.7	16.4	A
TD						
qTD-6 (6)	XNpb209–C688	2000	2.8	−1.7	17.6	A
		2001	2.8	−1.0	16.3	A
		2002	2.6	−1.5	14.6	A
qTD-8 (8)	G1149–R727	2000	2.5	2.6	16.0	I
		2001	5.9	1.9	30.4	I
		2002	3.3	1.4	14.5	I
VC						
qVC-4 (4)	C445–Ky4	2001	2.5	−1.0	18.9	A
qVC-6 (6)	XNpb209–C688	2001	3.7	−2.1	14.6	A
EL						
qEL-3 (3)	C1677–R19	2002	3.1	−1.2	17.2	A
qEL-8 (8)	G1149–R727	2002	2.3	0.8	11.9	I
IVOE						
qIVOE-6 (6)	XNpb209–C688	2000	2.9	−9.3	18.1	A
		2001	3.4	−5.1	17.5	A
		2002	2.7	−3.8	17.2	A
qIVOE-8 (8)	G1149–R727	2000	2.3	6.0	14.6	I
		2001	3.9	5.1	19.6	I
		2002	7.9	9.1	31.4	I
AC						
qAC-8 (8)	G1149–R727	2000	3.7	1.0	17.1	I
		2001	2.5	1.0	13.4	I
		2002	2.4	1.1	19.2	I
qAC-9a (9)	XNpb36–XNpb103	2001	2.5	1.4	8.0	I
qAC-9b (9)	C609–C506	2000	3.0	0.8	12.1	I
		2001	2.3	1.0	12.5	I
qAC-12 (12)	XNpb189-2–XNpb24-2	2000	2.3	0.8	8.2	I
		2002	5.0	1.2	21.0	I
GT						
qGT-3 (3)	C1677–R3156	2000	5.1	−1.9	20.5	A
		2002	3.3	−2.1	21.3	A
qGT-1 (1)	C955–C970	2000	2.8	−1.9	13.8	A
qGT-6 (6)	C688–R2171	2001	2.3	1.1	6.7	I
GC						
qGC-4 (4)	C445–Ky4	2000	4.9	−3.8	15.0	A
		2002	2.8	−9.2	13.0	A
qGC-6 (6)	XNpb209–C688	2000	2.3	−3.3	8.0	A
		2001	2.4	−4.4	9.6	A
qGC-11 (11)	XNpb257–C1350	2000	8.3	−9.3	32.0	A
		2002	4.1	−8.0	20.0	A
qGC-3 (3)	C1677–R3156	2001	2.4	4.3	12.0	I
qGC-5 (5)	C263–R3166	2000	4.9	3.5	19.0	I

[a]Both AE and PVE were estimated from mean trait values of individual CSSLs in the individual years
[b]*I* IR24 allele, *A* Asominori allele

mapped in the XNpb209–C688 region on chromosome 6, and *qTD-6* and *qIVOE-6* were simultaneously identified in 3 years. And five QTLs (*qLT-8*, *qST-8*, *qTD-8*, *qEL-8*, and *qIVOE-8*) were mapped in the G1149–XNpb41 interval on chromosome 8, of which *qLT-8*, *qTD-8*, and *qIVOE-8* were all repeatedly detected across 3 years. Additionally, three QTLs (*qLT-4*, *qST-4*, and *qVC-4*) were detected in the C455–Ky4 interval on chromosome 4 and QTL *qLT-3* in the C1677–R19 region on chromosome 3 with low repeatability (in only 1 year).

Four QTLs affecting AC were detected in which *qAC-8* was repeatedly detected in the G1149–R727 interval across 3 years, while *qAC-9a*, *qAC-9b*, and *qAC-12* were detected only in 1 or 2 years. For GT, three QTLs (*qGT-1*, *qGT-3*, and *qGT-6*) were identified in 1 or 2 years. Among five GC QTLs, *qGC-4*, *qGC-6*, and *qGC-11* were identified in 2 years, but *qGC-3* and *qGC-5* were detected in only 1 year.

Most positive effects were contributed by Asominori alleles (Table 4).

Effects of QTLs with high repeatability

Among 25 QTLs detected in 3 years, six QTLs (*qLT-8*, *qTD-6*, *qTD-8*, *qIVOE-6*, *qIVOE-8*, and *qAC-8*) were repeatedly detected across 3 years (Table 4). Four of these QTLs (*qLT-8*, *qTD-8*, *qIVOE-8*, and *qAC-8*) were all located in the G1149–XNpb41 interval. Three CSSLs ($CSSL_{49}$, $CSSL_{50}$, and $CSSL_{51}$) had this chromosome region substituted by IR24 alleles at G1149 and XNpb41 loci (Kubo et al. 1999). Another two QTLs (*qTD-6* and *qIVOE-6*) were both mapped in the XNpb209–C688 region. Similarly, three CSSLs ($CSSL_{26}$, $CSSL_{35}$, and $CSSL_{36}$) had this region substituted by IR24 alleles at C688 and XNpb209 loci (Kubo et al. 1999). Analyses of *t*-tests showed significant difference of phenotypic values between Asominori and the CSSL carrying any of the target QTL alleles (Table 5). The results indicated that the effects of these six QTLs were significant and repeatable in three successive years. However, the other 19 QTLs were environment-specific as their significant effects were only detected in 1 or 2 years.

Discussion

Sensory analysis is one of the most valid methods to study organoleptic properties and has recently been used for the taste description of fruits and vegetables (Esti et al. 1997; Brennan et al. 1997; Causse et al. 2001). In rice, high palatability is such an important factor for quality that sensory analysis should be incorporated into rice quality-improvement program. However, the complexity of the taste test and the limited rice grains in early generations, however, restrain the effective utilization of the sensory method. Therefore, it is very valuable to apply DNA markers closely linked to genes (QTLs) for fine palatability to select desirable plants at early stages of rice breeding. In this study, we found five non-environment-specific QTLs for the palatability of

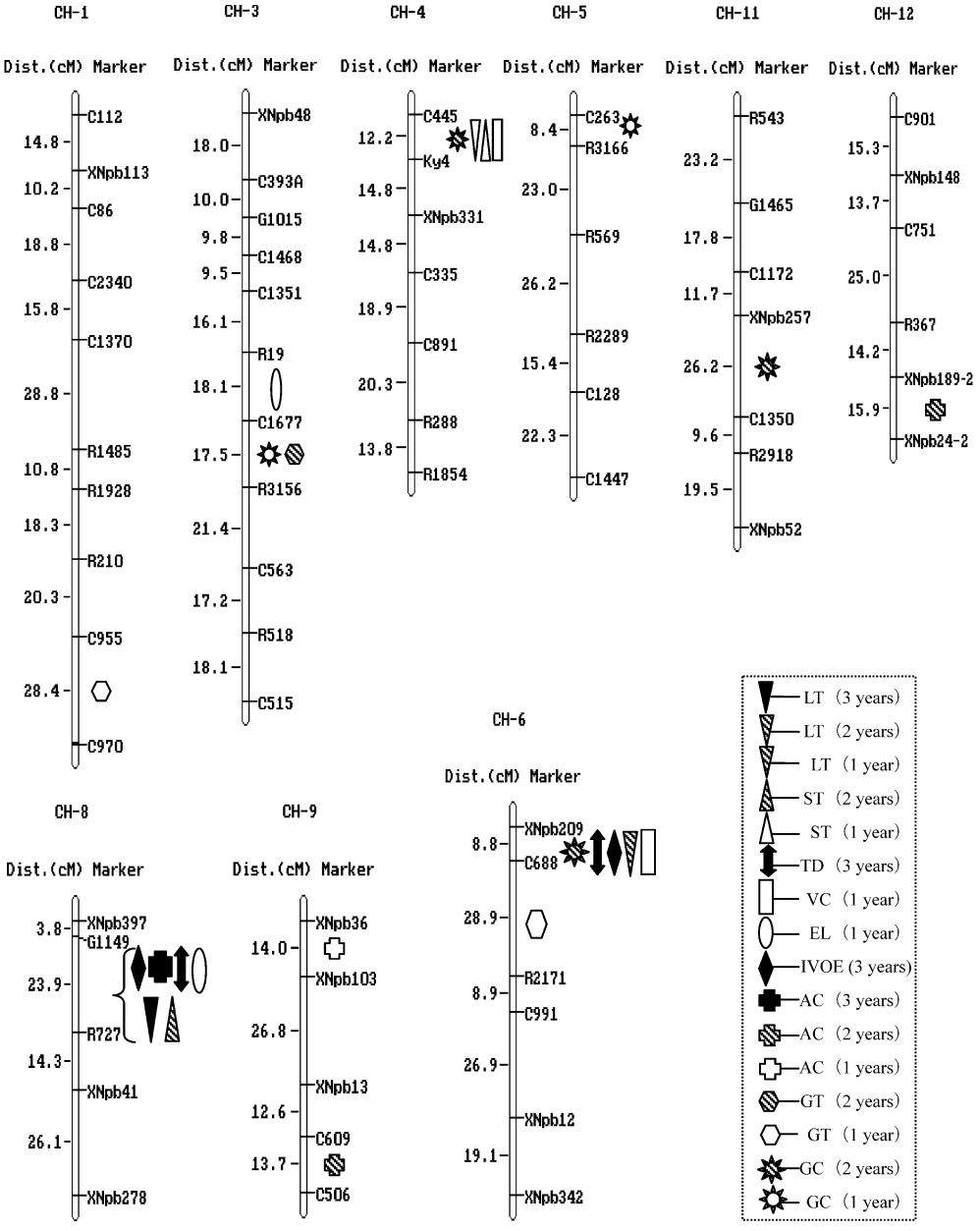

Fig. 2 Chromosomal locations of QTLs for the six palatability properties and three physico-chemical characteristics detected in individual years

cooked rice, which should be particularly helpful in marker-aided manipulation of rice eating quality.

Many QTLs affecting palatability and physico-chemical properties were mapped to the same genome regions (Fig. 2). Co-localization of these QTLs, as the results of either pleiotropic effects or close linkage, can provide an explanation for the genetic basis of correlations among the various quality traits. But the co-localization may occur just by chance, for example, the correlations were not significant between GC and any of the other traits controlled by the two QTL clusters in the C445–Ky4 and XNpb209–C688 intervals, though *qGC-4* and *qGC-6* were co-localized (Table 3; Fig. 2). The reasons presumably are the large number and the typical low resolution (5–15 cM) of QTLs detected (Xing et al. 2002; Wang et al. 1999; Li 2001). Therefore, further genetic studies, such as fine mapping of these QTLs, are required to determine the actual number of distinct QTLs in these regions and to elucidate the genetic mechanism of the co-localization. This work is underway by secondary F_2 populations derived from crosses between target CSSLs and Asominori.

It has been well known that AC is the key factor affecting rice eating quality, and it is mainly controlled by the *Wx* gene on chromosome 6 (Juliano 1985). However, no QTL affecting AC was detected at the *Wx* locus in this study, possibly because the difference of AC was relatively small between Asominori and IR24, which was in agreement with the result reported previously. Only five minor QTLs non-allelic to the *Wx* gene were

Table 5 *t*-Tests for the differences of phenotypic values between Asominori and the CSSL carrying any of QTL alleles with high repeatability in 3 years

Target QTLs and CSSLs	2000		2001		2002	
	Mean[a]	*P*-values	Mean[a]	*P*-values	Mean[a]	*P*-values
qTD-6						
Asominori	11.6		12.6		12.1	
$CSSL_{26}$[b]	5.4	0.025*	7.1	0.003**	6.1	0.000**
$CSSL_{35}$[c]	8.1	0.003**	9.1	0.006**	8.1	0.001**
$CSSL_{36}$[d]	7.8	0.007**	10.0	0.030*	8.2	0.002**
qIVOE-6						
Asominori	36.3		38.5		34.8	
$CSSL_{26}$[b]	17.1	0.000**	18.2	0.001**	17.4	0.001**
$CSSL_{35}$[c]	26.6	0.000**	25.3	0.004**	29.1	0.024*
$CSSL_{36}$[d]	23.6	0.000**	25.5	0.007**	25.4	0.006**
qLT-8						
Asominori	11.7		11.9		11.4	
$CSSL_{49}$	14.7	0.001**	14.8	0.002**	15.6	0.003**
$CSSL_{50}$	14.9	0.005**	13.6	0.019*	14.2	0.001**
$CSSL_{51}$	14.4	0.001**	14.3	0.002**	14.7	0.002**
qTD-8						
Asominori	11.6		12.6		12.1	
$CSSL_{49}$	15.4	0.008**	13.8	0.046*	14.3	0.001**
$CSSL_{50}$	14.7	0.047*	14.2	0.045*	14.3	0.003**
$CSSL_{51}$	15.7	0.003**	15.2	0.003**	14.6	0.006**
qIVOE-8						
Asominori	36.3		38.5		34.8	
$CSSL_{49}$	52.7	0.001**	49.3	0.007**	51.5	0.001**
$CSSL_{50}$	51.1	0.004**	44.5	0.049*	48.9	0.002**
$CSSL_{51}$	46.3	0.002**	48.2	0.010**	49.3	0.008**
qAC-8						
Asominori	14.6		15.1		15.3	
$CSSL_{49}$	16.2	0.022*	16.7	0.011*	17.9	0.036*
$CSSL_{50}$	17.0	0.010**	16.7	0.005**	16.8	0.016*
$CSSL_{51}$	16.8	0.010**	16.8	0.002**	18.8	0.028*

Significance levels: $*P \leq 0.05$, $**P \leq 0.001$

[a]Mean represents the trait average values of two replicates in field experiment

[b–d]The significant difference between Asominori and $CSSL_{26}$, $CSSL_{35}$, or $CSSL_{36}$ in 2000 at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.01$, respectively

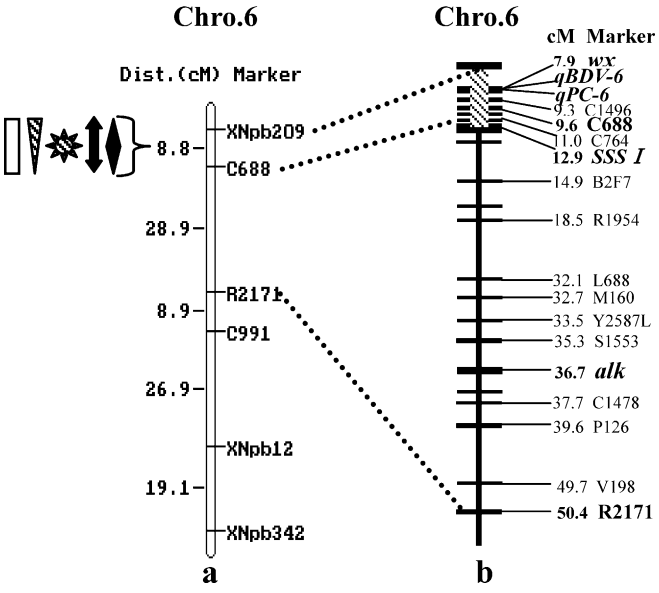

Fig. 3 Location relationship among eating quality-related QTLs in the XNpb209–C688 interval, genes encoding rice starch synthase, and QTLs for rapid viscosity analyzer profile and protein content. **a** The present study (Fig. 2), **b** Harushima et al. (1998), Tan et al. (2001), Bao et al. (2000), and Tanaka et al. (1995)

responsible for AC variation when a RILs population, derived from the cross between two low AC parents, Asominori and IR24, was applied to map QTLs (Wu et al. 2000). Therefore, it is understandable that four QTLs identified for AC in the present study were not mapped near the *Wx* gene since the parents here were also Asominori and IR24.

However, the QTL cluster on chromosome 6 was located in the same region as the *Wx* gene (Fig. 3), and this cluster played an important role in underlying the palatability of cooked rice. The *Wx* gene not only encodes the granule-bound starch synthase which produces amylose in non-glutinous rice kernels (Sano 1984), but also affects the structure of amylopectin which is of prime importance to rice eating quality (Villareal et al. 1997; Ball et al. 1998). On the other hand, the Wx^a allele is present in the *indica* parent IR24, but the Wx^b allele is in the *japonica* parent Asominori (Sano et al. 1986), which leads to the single-nucleotide change and functional differences of the *Wx* alleles between Asominori and IR24 (Wang et al. 1995; Ayres et al. 1997). Therefore, the differences of structure and function of *Wx* alleles may result in the different structures of amylopectin without significant AC variation between Asominori and IR24. Additionally, many genes (QTLs) for synthesis of amylopectin, protein content (PC), and starch breakdown viscosity (BDV) were also located in the XNpb209–C688 interval (Fig. 3). These may be the genetic mechanism by which this region on chromosome 6 played an important role in underlying eating quality.

Four QTLs, *qLT-8*, *qTD-8*, *qIVOE-8*, and *qAC-8*, were repeatedly detected in the G1149–XNpb41 region in 3 years (Fig. 2). In addition, Jiang (2002) and Hu (2002) mapped the *SSSIII* gene for synthesis of amylopectin and QTL *qPAL-8a* for palatability (using automatic taste analyzer) in the V115–R1813 and R1813–C1121 intervals on chromosome 8, respectively. Since the two regions were overlapping or in the same region as the G1149–XNpb41 region (Fig. 4), the QTL cluster on chromosome 8 was also the key genetic factor for the palatability of cooked rice.

The CSSLs harboring desirable QTL alleles have been backcrossed to Asomimori, and six secondary F_2 populations are being constructed for fine mapping those major and non-environment-specific QTLs alleles. The objectives of the further research are to obtain DNA markers tightly linked to the desirable QTLs and to facilitate MAS in rice breeding for high quality, and to separate the key gene on chromosome 8 by the strategy of positional cloning since the *SSSIII* gene has not been cloned so far.

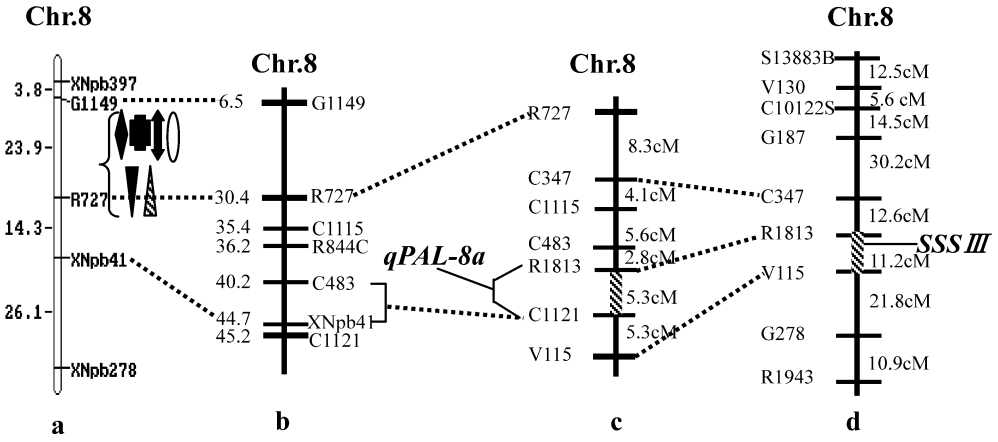

Fig. 4 Location relationship among *qPAL-8a* genes encoding soluble starch synthase and eating quality-related QTLs in the region of G1149–XNpb41 on chromosome 8. **a** The present study (Fig. 2), **b** Tsunematsu et al. (1996), **c** Harushima et al. (1998) and Hu (2002), **d** Jiang (2002)

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